Safety Profile of the Merck Human Immunodeficiency Virus-1 Clade B *gag* DNA Plasmid Vaccine With and Without Adjuvants

Erin K. Quirk,[1,a] Elizabeth L. Brown,[1] Randi Y. Leavitt,[1] Robin Mogg,[1] Devan V. Mehrotra,[1] Robert K. Evans,[1] Mark J. DiNubile,[2] and Michael N. Robertson[1]

[1]Merck Research Laboratories, West Point, Pennsylvania; [2]Office of the Chief Medical Officer, Merck, Upper Gwynedd, Pennsylvania

The immunogenicity results from 3 phase I trials of the Merck DNA human immunodeficiency virus (HIV) vaccine have previously been reported. Because preventive DNA vaccine strategies continue to be leveraged for diverse infections, the safety and tolerability results from these studies can inform the field moving forward, particularly regarding adverse reactions and adjuvants. No serious vaccine-related adverse events were reported during the 3-dose priming phase. Pain at the injection site was more common with adjuvanted formulations than with the phosphate-buffered saline diluent alone. Febrile reactions were usually low grade. Although the $AlPO_4$ or CRL1005 adjuvants used in these studies did not significantly enhance the immunogenicity of the DNA vaccine, adverse events were numerically more common with adjuvanted formulations than without adjuvants.

Keywords. adjuvants; DNA plasmid vaccine; HIV; safety.

DNA-vaccine strategies continue to be explored for the prevention of human immunodeficiency virus (HIV), influenza, respiratory syncytial virus, cytomegalovirus, hepatitis C virus, and other viral, bacterial, and parasitic infections [1–5] as well as for noninfectious diseases [6, 7]. Adjuvants can augment cell-mediated immune responses against some vaccine targets. Traditional adjuvants based on aluminum compounds are thought to act as depots, slowing absorption of antigen while recruiting inflammatory cells, activating complement, and affecting particle size to facilitate uptake of antigen by antigen-presenting cells. A newer group of adjuvants based on nonionic block copolymer compounds had been used extensively as pharmaceutical excipients and later developed as potential vaccine adjuvants. CRL1005, originally developed by CytRx Corp (Norcross, GA), is a linear triblock copolymer with a polyoxypropylene core of molecular weight 12 kDa and 5% polyoxyethylene. Potential mechanisms of action include direct stimulation of macrophages and indirect activation of the complement cascade. In animal models and clinical trials, copolymer adjuvants such as CRL1005 have been generally well tolerated while variably enhancing cell-mediated immune responses to some vaccines [8]. In preclinical testing, both the $AlPO_4$ and CRL1005 adjuvants augmented antigen-specific immune responses against simian immunodeficiency virus induced by a DNA-plasmid vaccine in rhesus macaques [9]. Because a prime-boost strategy initiated with a DNA vaccine is still an area of active research, unpublished information regarding the safety and tolerability of the discontinued Merck HIV-1 DNA-plasmid vaccine with and without an adjuvant during the 3-dose priming series remains relevant to the field [8–10].

Healthy HIV-seronegative adults were enrolled in 3 independent dose-escalating, blinded, placebo-controlled phase I trials evaluating the safety and immunogenicity of a 3-dose homologous priming regimen of the Merck HIV-1 *gag* vaccine containing 1 mg or 5 mg DNA per 1 mL dose formulated as a sterile solution of phosphate-buffered saline ([PBS] 6 mM sodium phosphate, 150 mM NaCl, pH 7.2) with or without either $AlPO_4$ (0.7 mg/mL) or CRL1005 (7.5 mg/mL) with 0.6 mM benzalkonium chloride [8]. Placebos consisted of an identical diluent with or without 0.7 mg AlPO4/mL. Subjects received priming doses of the DNA-plasmid at weeks 0, 4, and 8, followed by a booster at week 26 with either the DNA or adenovirus-type 5 vaccine. The 1.0 mL dose of vaccine or placebo was to be injected intramuscularly using an appropriately sized needle based on the clinician's judgment at a 90° angle into the deltoid. All injections were to be administered in the same arm. Local and systemic adverse events were respectively monitored for at least 5 and 15 days following each intradeltoid injection.

Of 360 randomized subjects, 359 (99.7%) received ≥1 injection and were included in the analyses, and 343 (95.3%) completed the full 3-dose priming series (Table 1). Predominantly young white adults with a slight preponderance of men over women entered the study. Unfractionated *gag*-specific

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[a]Present affiliation: Gilead Sciences, San Francisco, CA.

Correspondence: Michael Robertson, MD, UG3D-011, PO Box 1000, North Wales, PA 19454-1099. (michael_robertson@merck.com).

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Table 1. Baseline Characteristics by Treatment Group During the Priming Phases of the Combined Studies

	1 mg DNA/ PBS (N = 42)	5 mg DNA/ PBS (N = 74)	5 mg DNA/ $AlPO_4$ (N = 78)	5 mg DNA/ CRL 1005 (N = 89)	Placebo/PBS (N = 41)	Placebo/$AlPO_4$ (N = 35)
Gender (n [%])						
Male	26 (62)	38 (51)	48 (62)	53 (60)	24 (59)	17 (49)
Female	16 (38)	36 (49)	30 (38)	36 (40)	17 (41)	18 (51)
Age (years)						
Median	34	37	32	37	39	37
Range	18–55	19–53	18–50	20–50	18–55	18–50
Asian/Pacific	1 (2)	0 (0)	4 (5)	2 (2)	0 (0)	0 (0)
Racial/Ethnic Origin (n [%])						
Black	5 (12)	7 (9)	4 (5)	6 (7)	4 (10)	3 (9)
Caucasian	34 (81)	63 (85)	67 (86)	78 (88)	35 (85)	31 (89)
Hispanic	2 (5)	3 (4)	2 (3)	2 (2)	1 (2)	1 (3)
Indian	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Native American	0 (0)	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)
Multiracial	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)

Abbreviations: N, number of vaccinated subjects; n, number of subjects in each category; PBS, phosphate-buffered saline.

interferon-γ enzyme-linked immunosorbent spot (ELISPOT) responses were more robust after the 5 mg than the 1 mg DNA-dose but were not significantly enhanced by the addition of either adjuvant. Postprime response rates (predefined as the proportion of subjects with ≥55 spot-forming cells per 10^6 peripheral blood mononuclear cells with at least a 4-fold increase over control at week 12 [4 weeks after the last priming dose]) for the unadjuvanted vaccine formulation (21.9%) trended modestly higher than for the CRL1005-adjuvanted vaccine (16.0%) and essentially the same as the $AlPO_4$-adjuvanted vaccine (22.5%) [8]. Postprime geometric-mean ELISPOT titers were likewise similar across the 3 formulations, ranging from 67 to 79 spot-forming cells.

No serious vaccine-related adverse events were reported. No subject discontinued participation during the priming phase because of vaccine-related adverse events. Local reactions were more common than systemic side-effects. Pain at the injection site occurred more often with adjuvanted formulations than with the PBS diluent, whether given with or without DNA. Despite symptomatic differences, local signs of inflammation developed with generally similar frequency in the presence or absence of adjuvant. The proportions of subjects with injection site reactions in each treatment group remained fairly constant after each of the 3 priming doses. The rates of systemic adverse events were generally comparable in the vaccine and placebo groups, although the incidence of headache and fatigue were modestly higher in the presence than absence of adjuvant [10]. Febrile reactions were mostly low grade, usually <38°C. Although the addition of $AlPO_4$ or CRL1005 adjuvants did not appear to enhance immunogenicity of the DNA vaccine, adverse reactions were numerically more common with adjuvanted formulations than without adjuvants.

In summary, no serious adverse reactions were attributed to the Merck HIV-1 *gag* vaccine whether administered with or without an adjuvant. Both local and systemic side-effects were generally mild. Injection-site pain was more frequent and prominent in recipients of adjuvanted formulations, even when given as part of the placebo. Because the adjuvants used in these studies did not produce a discernable immunological benefit but negatively impacted the local tolerability of the vaccine [8, 10], routine inclusion of these adjuvants in future DNA-vaccine trials does not appear to be prudent unless persuasive evidence of their utility with a particular vaccine exists [11]. Novel adjuvants are under development [12, 13].

Notes

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